Attorney Docket No. 36609-300901

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	)	
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R. Paul Schaudies, et al.	)	
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Serial No. **10/630,384**	)	Art Unit: **1637**
	)	
Filed: **JULY 30, 2003**	)	Examiner: **FREDMAN, J.**
	)	
For: **METHOD FOR DETECTING A BIOLOGICAL ENTITY IN A SAMPLE**	)	
	)	

DECLARATION BY R. PAUL SCHAUDIES UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1430

Sir:

I, R. Paul Schaudies, Ph.D., do hereby declare:

1. I received a Doctor of Philosophy degree in Biochemistry from Temple University School of Medicine in 1985. Since receiving my Ph.D., I have worked as a Chief Investigator in the Department of Clinical Investigation at Walter Reed Army Medical Center in Washington, D.C.; worked as a Senior Scientist in the Department of Nephrology at the Walter Reed Army Institute of Research in Washington, D.C.; and worked as a Chief Investigator in the Central Measurement and Signature Intelligence Office, Chemical and Biological Collection Systems Division, at the Defense Intelligence Agency in Washington, D.C. I am currently Assistant Vice President and Division Manager for Biological and Chemical Defense at Science Applications International Corporation. In this capacity, I spearheaded development of the procedures for the *Bacillus anthracis* decontamination of several federal buildings in Washington, D.C. after the anthrax attack of 2001.

2. I am an inventor of U.S. Patent Application Serial No. 10/630,384, filed July 30, 2003, entitled "Method for Detecting a Biological Entity in a Sample." I am familiar with the application and the rejections in the Office Action issued by the Examiner on June 17, 2005.

3. The following experiments were performed under my supervision. These experiments demonstrate that successful and unexpected results are obtained in performing the methods claimed in the above-referenced patent application.

Experiment 1

This experiment was designed to demonstrate that the methods described in the above-reference patent application are highly specific and generate numerous redundancies that result in minimal false positives and, therefore, a high level of confidence.

Nucleic acid sequences were obtained from *Bacillus anthracis* (Sterne), *B. anthracis* (Ames), *B. cereus*, *B. thurigiensis*, *Francisella tularensis*, *Yersinia pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. Genomic DNA from each of the bacteria, as well as human DNA, were combined with multiple primers of randomized nucleotide sequences. A detectable nucleoside triphosphate, labeled with cy3, was incorporated during amplification to produce detectable amplification products. The detectable amplification products were then hybridized to nine identical arrays. Predetermined oligonucleotides specific to each species were arranged in a banding pattern on the array to facilitate visualization of the data without bioinformatics analysis. Images are intentionally over-exposed to enhance visualization. A color coded map of the array and a key are presented for identification.

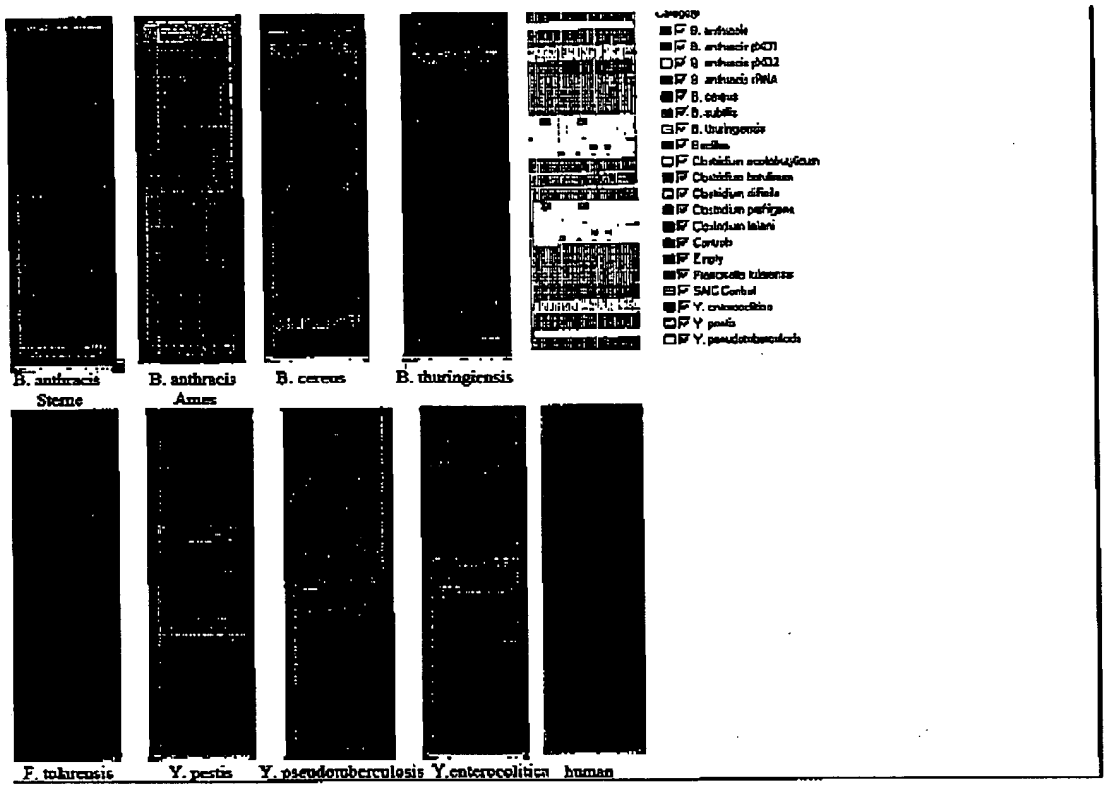

Figure 1. Detection of multiple genomes on identically spotted arrays.

Figure 1 shows that labeled amplification products generated for each bacterial species of interest hybridized to their corresponding oligonucleotides on the array with minimal cross reactivity with oligonucleotides specific to other bacterial species. Accordingly, Figure 1 demonstrates that a target biological entity can be detected with high specificity in a sample using the random primer amplification methods claimed in the above-referenced patent application without cross-reactivity from non-target DNA.

Figure 1 further demonstrates the increased confidence with which a particular biological entity can be detected. The amplification step allows for essentially an entire target genome of interest to be amplified. This, combined with the printing of pre-

determined oligonucleotides that represent multiple sites in the target genome, allows for the generation of multiple positive signals. This increased redundancy results in a dramatic increase in the confidence with which a particular biological entity is detected and decreases the generation of false positives and false negatives. These aspects of the above-referenced patent application can be further appreciated in the figures that follow.

Figure 2 demonstrates that not only can the above method be used to distinguish between multiple species it can further differentiate between variants of a single species with a high degree of confidence. The Sterne variant of *B. Anthracis* lacks the pXO2 virulence plasmid found in the Ames variant. Both variants contain the pXO1 virulence plasmid. Nucleotide sequences from both variants were amplified and labeled according to the protocol used in the initial experiment. The array contains oligonucleotides identified from unique chromosomal regions of the genome as well as the virulence plasmids pXO1 and pXO2. A color coded map used to determine the identity of the oligonucleotides is located in the center of the image. Red represents unique chromosomal regions, blue is pX01 and yellow is pX02. The array on the left was hybridized with *B. anthracis* (Sterne) so the pXO2 spots do not hybridize. The array on the right was hybridized with *B. anthracis* (Ames) and the pXO2 spots do hybridize. The five dark spots in the Sterne hybridization represent chromosomal sequences absent in Sterne but present in Ames.

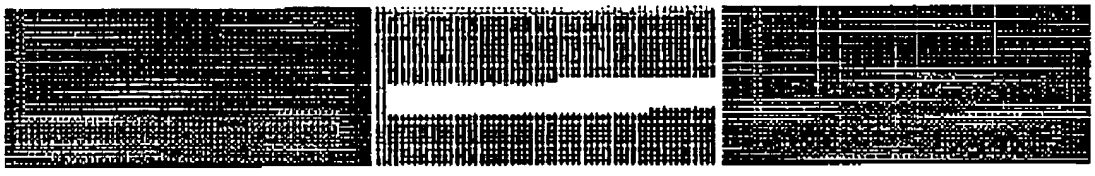

Figure 2. Hybridization patterns between two variants of *Bacillus anthracis*.

Figure 3 provides a graphical representation of the above data. Each data point in the scatter plot is a function of its hybridization intensity in two separate hybridizations. The "X" value is the intensity with Sterne and the "Y" value with Ames. Sequences present in both strains fall along the Y=X line. As can be seen, most of the data points lie along the Y=X line between these two closely related species. However, the use of multiple oligonucleotides specific to pXO2 allow the Ames strain to confidently be differentiated from the Sterne strain.

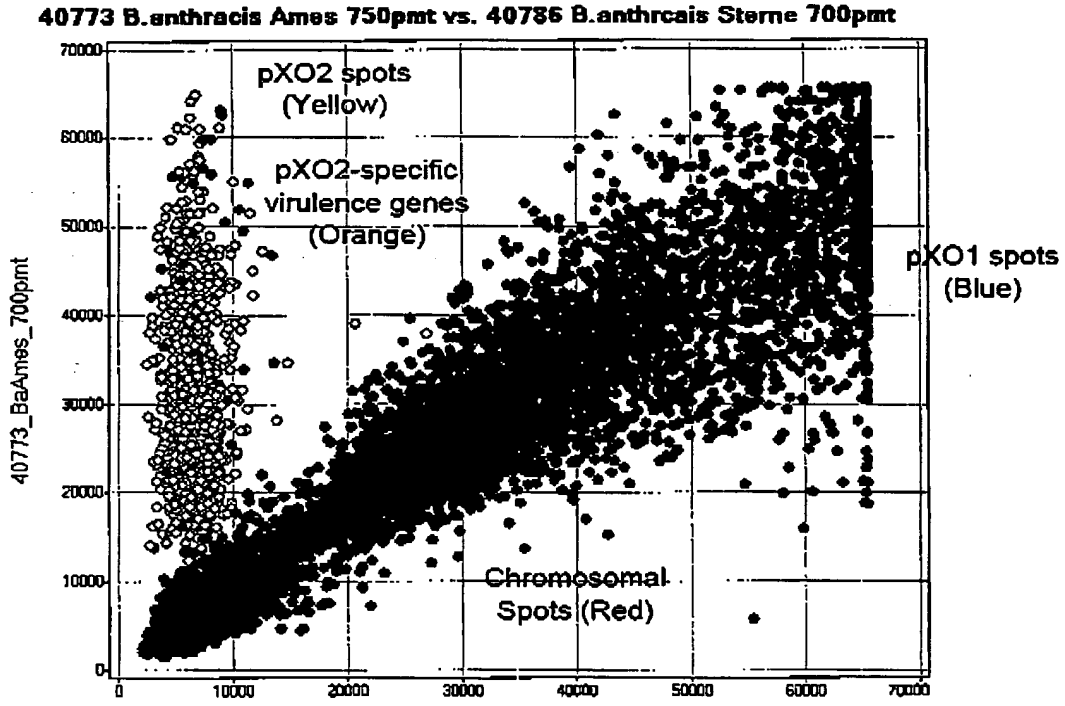

Figure 3. Scatter plot of hybridization intensities: *B. anthracis* (Ames) vs. *B. anthracis* (Sternes).

The data provided above can be contrasted with Figure 4, which demonstrates a scatter plot comparing the hybridization intensities between two different species. Each data point in the scatter plot below is a function of its hybridization intensity in two separate hybridizations. The "X" value is the intensity with *B. anthracis* (Sterne) and the

"Y" value with *B. thuringiensis* 97-27. Sequences present in both strains fall along the Y=X line. The green spots on the Y=X line represent chromosomal sequences present in both species. The black spots on the Y=X line represent Bacillus-specific sequences incorporated into the array design. Figure 3 shows that the data points generated using the method of the above-referenced patent application can easily distinguish between the two species.

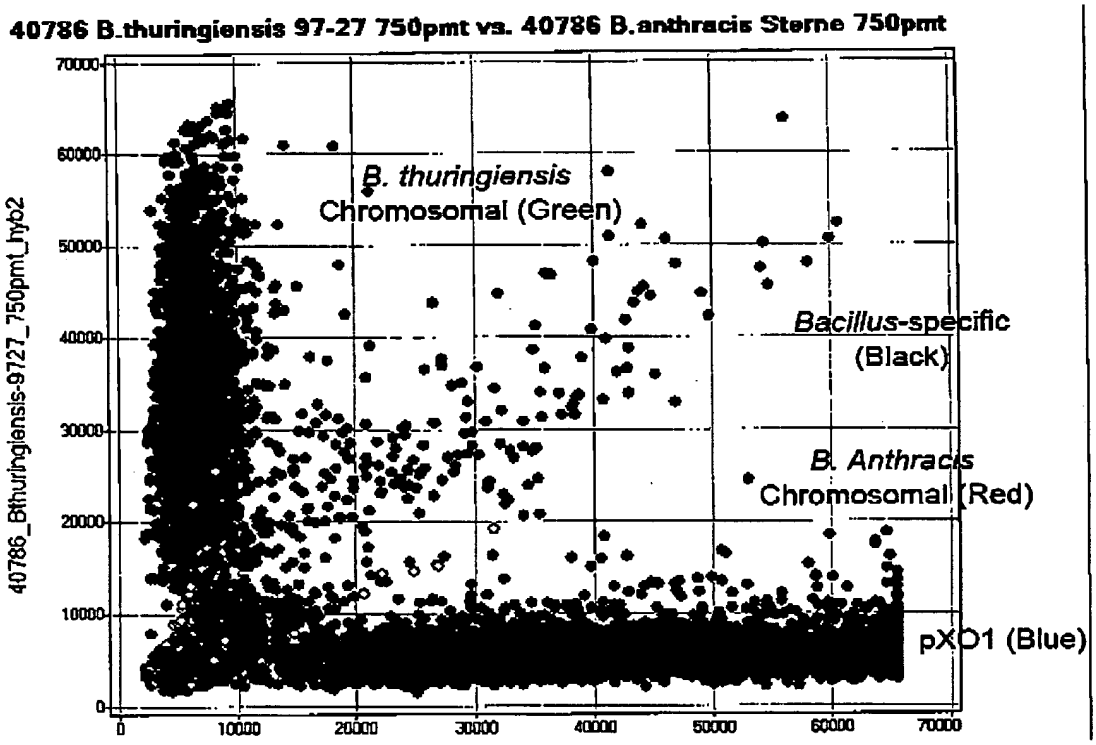

Figure 4. Scatter plot of hybridization intensities: *B. thuringiensis* vs. *B. anthracis* (Sterne)

4. The experiments described above demonstrate that the methods of the above-referenced patent application can be used to amplify, label and fragment essentially the entire genome of a biological entity for microarray analysis, which results in a significant increase in the redundancy of signal as compared to currently available

methods. This increase in the redundancy of signal results in a dramatic increase in the confidence with which a particular biological entity is detected. Such results cannot be achieved by practicing the methods found in the prior art references cited by the Examiner, thereby showing that methods described in the above-referenced patent application are not obvious in view of these prior art references.

5. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patent issuing on this application.

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Date

R. Paul Schaudies